Comparison of ABF1 and RAP1 in Chromatin Opening and Transactivator Potentiation in the Budding Yeast *Saccharomyces cerevisiae*

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Autonomously replicating sequence binding factor 1 (ABF1) and repressor/activator protein 1 (RAP1) from budding yeast are multifunctional, site-specific DNA-binding proteins, with roles in gene activation and repression, replication, and telomere structure and function. Previously we have shown that RAP1 can prevent nucleosome positioning in the vicinity of its binding site and have provided evidence that this ability to create a local region of "open" chromatin contributes to RAP1 function at the *HIS4* promoter by facilitating binding and activation by GCN4. Here we examine and directly compare to that of RAP1 the ability of ABF1 to create a region of open chromatin near its binding site and to contribute to activated transcription at the *HIS4*, *ADE5,7*, and *HIS7* promoters. ABF1 behaves similarly to RAP1 in these assays, but it shows some subtle differences from RAP1 in the character of the open chromatin region near its binding site. Furthermore, although the two factors can similarly enhance activated transcription at the promoters tested, RAP1 binding is continuously required for this enhancement, but ABF1 binding is not. These results indicate that ABF1 and RAP1 achieve functional similarity in part via mechanistically distinct pathways.

Autonomously replicating sequence (ARS) binding factor 1 (ABF1) and repressor/activator protein 1 (RAP1) are multifunctional proteins expressed in the budding yeast *Saccharomyces cerevisiae* that have both been categorized as general regulatory factors (GRFs) (3). Both proteins play important roles in transcriptional activation and repression, gene silencing, recombination, and telomere structure, and both are abundant and essential for cell growth (12, 39, 45). Binding sites for both proteins are present in the promoter regions of numerous yeast genes, and ABF1 and RAP1 have been shown to contribute to transcriptional activation of genes involved in carbon source regulation, sporulation, amino acid biosynthesis, and ribosomal functions (21, 32, 33, 45). ABF1 and RAP1 act in concert to prevent gene expression at the silenced mating-type loci (7). ABF1 has also been implicated in gene silencing within subtelomeric regions (35) and nucleotide excision repair of silenced chromosomal regions (36), whereas RAP1 binds directly to telomere repeats to initiate formation of heterochromatin-like telomere structures (11).

Given their overlapping functions, it is not surprising that in several specific instances ABF1 and RAP1 have been found to be directly interchangeable. Both RAP1 and ABF1 can synergize with T-rich elements present in the rp*S33* and rp*L45* promoters to activate transcription (8), and they have also been reported to be interchangeable at the *TRP3* promoter (23). Both RAP1 and ABF1 binding sites in ribosomal protein gene promoters are associated with recruitment of Esa1p and concomitant histone acetylation, as well as with recruitment of TFIID (24, 37). Furthermore, binding sites for RAP1 and

ABF1 support *ARS1* replication origin function equally well in a plasmid stability assay (22), and both proteins can function as insulator elements to establish boundaries between regions of silent (heterochromatic) and permissive (euchromatic) chromosomal regions (6, 60). Finally, both ABF1 and RAP1 possess functionally important C-terminal regions (10, 13, 26). Although these domains have only limited sequence homology (5), they are functionally interchangeable in cell viability assays and partly interchangeable in supporting transcriptional activation with specific promoters (9).

The precise mechanisms by which RAP1 and ABF1 contribute to the multiple processes in which they function are still being elucidated. Correspondingly, the basis for their functional similarity is not yet fully understood. One means by which ABF1 and RAP1 might contribute to disparate processes is by opening chromatin to allow access by other proteins (3, 4, 16, 19, 23, 29, 40, 58). This mode of action would also be consistent with findings that ABF1 and RAP1 are not able to stimulate robust transcription by themselves but synergize strongly with other transcription factors (4, 8, 23, 40, 42). Furthermore, direct observations show that both ABF1 and RAP1 can remodel chromatin near their binding sites, and RAP1 can outcompete histones for occupancy of its binding site (2, 16, 26, 54, 58, 59).

Although ABF1 and RAP1 are functionally similar, they differ in some regards. The two proteins share only a limited region of homology in their essential C-terminal regions. Both proteins have distinct DNA-binding domains and C-terminal regions that are functionally important. However, the C-terminal region of RAP1 contains domains that interact with various proteins involved in telomere and mating-type silencing, whereas interactions involving the ABF1 C-terminal region have not yet been identified, although regions important

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to transcription, chromatin remodeling, and viability have been mapped (26). The overall lack of homology in the functionally important C-terminal regions suggests that ABF1 and RAP1 may differ in some of the mechanistic aspects by which they perform their disparate functions. Furthermore, although ABF1 and RAP1 are equally effective at providing replication origin function at *ARS1*, replacement of the naturally occurring ABF1 binding site at *ARS121* by a RAP1 binding site results in reduced replication efficiency, as assessed by mitotic stability of an *ARS121*-containing plasmid (22, 56).

Another indication that ABF1 and RAP1 may differ in the mechanism by which they function comes from experiments examining whether ABF1 or RAP1 binding is continually required to support activated transcription in which they participate. Using an *abf1-1 ts* mutant, Schroeder and Weil showed that, at a number of promoters requiring ABF1 sites to support normal transcriptional levels, little or no decrease in mRNA levels was seen at the restrictive temperature, although ABF1 binding was unambiguously lost (44). In contrast, RAP1 binding is continuously required at the *HIS4* promoter to support normal levels of GCN4-mediated transcription (59).

Here, we directly compare ABF1 and RAP1 function in perturbing chromatin via nucleosomal binding sites, in synergizing with other transcription factors in transcriptional activation, and in whether their continuous binding is required to support activated transcription. Our results support the idea that these GRFs both perform their multiple tasks in part by a strong ability to perturb chromatin structure, and they possess very similar capabilities to synergize with other transcription factors in activating transcription. However, we also uncover differences between these two proteins that point to their using distinct mechanisms to support activated transcription.

MATERIALS AND METHODS

Plasmids. The yeast plasmids TA/GCN1Δ80 and TAR/GCN1Δ80 are derived from the *TRP1ARS1* plasmid and have been described previously (58). To create TAA/GCN1Δ80, the consensus ABF1 binding site was first inserted into pRS104-GCN1Δ80 (58) adjacent to the GCN4 binding site by two-step PCR (14) with primers A and B (Table 1) to create pRS104-A/GCN1Δ80, which was verified by DNA sequencing. Yeast DNA sequence was excised from this plasmid by SacI and HindIII, ligated with the complementary SacI-HindIII fragment of pRS110 (30), and then transformed into yeast (15) to create TAA/GCN1Δ80. Transformants were verified by Southern analysis. Similarly, TAAmut/GCN1Δ80, created with primers C and D (Table 1), contains a mutated ABF1 binding site (40) adjacent to the GCN4 binding site.

To construct *HIS4-MEL1* reporter plasmids having a wild-type or mutant RAP1 binding site, the *HIS4* promoter was amplified from genomic DNA from yeast strain LYY596 or LYY599, respectively (58), by use of primers shown in Table 1. These PCR products were cloned as BamHI-SacI fragments into 416-*MEL1* (41), a plasmid that has a *URA3* marker and the *MEL1* coding sequence, to create 416-*HIS4-MEL1*. The *HIS4* promoter sequences were confirmed by sequencing. Plasmids that contain modified *HIS4* promoters with a wild-type or mutant ABF1 binding site in place of the RAP1 binding site were constructed by PCR amplification by use of primers shown in Table 1.

To construct reporter plasmids containing *HIS7* and *ADE5,7* promoters containing wild-type or mutant RAP1 or ABF1 binding sites fused to the *MEL1* coding sequence, we first amplified promoter sequences from genomic DNA by using primers listed in Table 1. The resulting PCR products were cloned into pRS416-*MEL1* (41) with the use of SacI and BamHI restriction sites. Construction of reporter plasmid variants in which the ABF1 binding site in *HIS7* or *ADE5,7* is replaced by a mutant ABF1 site, or a wild-type or mutant RAP1 binding site, was done by PCR with the use of primers shown in Table 1. Promoter sequences of these reporter plasmids were confirmed by DNA sequencing.

Strains and media. The *S. cerevisiae* strains used in this study are listed in Table 2. Strains harboring *ABF1* deletion mutations or the *abf1-1 ts* mutation were made by introducing the appropriate *ABF1* gene in the pRS315 plasmid into TMY86, as described previously (26). Strains AY(1-731) and AY(1-592) were generated from strains 1-731 and 1-592 by replacement of the *GCN4* open reading frame with the KanMX selectable marker. Yeast cells were grown at 30°C, unless stated otherwise, in complete synthetic medium (Bio 101) containing 2% glucose. Cell transformations were performed using a standard lithium acetate method (15).

Analysis of chromatin structure. Yeast cells were grown at 30°C to a density at 600 nm of between 0.6 and 1.3. Yeast spheroplast lysates were prepared and digested with micrococcal nuclease (MNase) and were analyzed by the indirect end-label technique as described elsewhere (17, 49). At least two independent transformants of each strain were tested.

α-Galactosidase assay. α-Galactosidase activity was measured as described previously (41). Three independent clones were assayed for each reported value, and each experiment was repeated at least two times. Error bars shown in the figures represent standard deviations.

Northern analysis. For Northern analysis involving temperature shift (see Fig. 6), cells were grown at 25°C overnight. The cultures were mixed with equal amounts of fresh medium preequilibrated to 25°C for analysis of cells grown at 25°C or to 50°C for analysis of cells shifted to 37°C. RNA was extracted from yeast cells, and approximately 8 to 10 μg was electrophoresed on agarose gels containing formaldehyde. Gels were blotted onto nylon membranes in 10× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate), UV cross-linked, and hybridized with probes labeled by random priming. Blots were stripped by boiling membranes in 0.015 M NaCl–0.1× SSC–1% sodium dodecyl sulfate before hybridization with another probe. Northern blots were quantitated using scanned images on a Molecular Dynamics PhosphorImager. The KpnI fragment from *HIS7-MEL1* containing most of the *MEL1* sequence was used as a *MEL1* DNA probe. The BglII fragment of *pGEM-PYK1* was used as a *PYK1* DNA probe.

ChIP. For chromatin immunoprecipitation (ChIP) involving temperature shift, cells were grown at 25°C overnight. The cultures were mixed with equal amounts of fresh medium preequilibrated to 25°C for analysis of cells grown at 25°C or to 50°C for analyzing cells shifted to 37°C for 1 h. ChIP was performed (see Fig. 7) essentially as described elsewhere (50), with the use of 20 μl of ABF1 antibody (SC6679; Santa Cruz Biotechnology). Cross-linked samples were sonicated using three repetitions of six pulses at 90% duty cycle, 20% output with a sonifier (model 250; Branson Ultrasonics, Danbury, Conn.), resulting in fragments ranging from 0.2 to 1.0 kb. Immunoprecipitated (IP) samples were eluted using standard protocols. For analysis, typically 1 to 2 μl of IP DNA or 1 μl of a 1:100 dilution of input DNA was amplified by using primers for *HIS4*, *SPT15*, and *ACT1* (as negative control) (Table 1). PCR amplification was performed for 22 and 26 cycles, and aliquots were electrophoresed, Southern blotted, and hybridized to verify that amplification was in the linear range. We found that multiplexing primers used for analysis of *SPT15*, *PYK1*, and the modified *HIS4* promoter resulted in spurious products that made quantitation difficult. We therefore performed amplification for each fragment in separate reactions and mixed aliquots from the separate reaction mixtures prior to gel electrophoresis. To guard against pipetting errors, each reaction was performed and analyzed at least three times, for two independent ChIP experiments. Quantitation was performed by using a Molecular Dynamics PhosphorImager.

RESULTS

ABF1 perturbs nucleosome positioning via a nucleosomal binding site similarly to RAP1. Previous work has indicated that one role of RAP1 is to assist activator binding by opening chromatin (4, 42, 58). Consistent with this idea, we have shown that RAP1 can perturb chromatin structure via a nucleosomal binding site in yeast (58). ABF1 is another general regulatory factor that shares many functions with RAP1 (see introduction). ABF1 has been shown to alter nucleosome positioning in the vicinity of a nonnucleosomal binding site in yeast (2, 16, 19, 54); however, a direct comparison of the ability of RAP1 and ABF1 to remodel chromatin has not been made, nor has the ability of ABF1 to perturb chromatin via a nucleosomal binding site been tested. To compare directly the abilities of RAP1 and ABF1 to perturb chromatin, we constructed four yeast

TABLE 1. Primers used in this study

Purpose	Primer	Restriction site created
Introduce wild-type ABF1 binding site in TA/GCN1Δ80	(A) 5′-CGATCCGTCGGTAGTGACTTTTATGCTTGGTTTTCC (B) 5′-GTCACTACCGACGGATCGATGACTATAAAAC	SphI
Introduce mutated ABF1 binding site in TA/GCN1Δ80	(C) 5′-CGATCCGGTGGTAGTGACTTTTATGCTTGGTTTTC (D) 5′-GTCACTACCACCGGATCGATGACTCATAAAAC	SphI
Introduce wild-type ABF1 binding site into *HIS4* promoter	(E) 5′-ATTGCCGTCGGTAGTGACGGCATGCACAGTGACTCACG (F) 5′-GTCACTACCGACGGCAATTAATTAACTAATTTACCGGAGTC	SphI
Introduce mutated ABF1 binding site into *HIS4* promoter	(G) 5′-ATTGCGGTCGGTAGTGACGGCATGCACAGTGACTCACG (H) 5′-GTCACTACCACCGGCAATTAATTAACTAATTTACCGGAGTC	SphI
Introduce SacI site into *HIS4* promoter	(I) 5′-CAATTGG AGCTCGAACGCAGAC	SacI
Introduce BamHI site into *HIS4* promoter	(J) 5′-TTTGGATCCTATTGTATTACTATTACACAGCGCAG	BamHI
Introduce SacI site into *HIS7* promoter	(K) 5′-CGCGATGAGCTCTGATTGACTACTCTCACGGTAACTCC	SacI
Introduce BamHI site into *HIS7* promoter	(L) 5′-GCCATGGATCCTCTCTTTTTCTTTACTTGTAAATAATTAAAAACC	BamHI
Introduce RAP1 binding site into *HIS7* promoter	(M) 5′-CATGATCACCTAATTTGTGCATGGGTTTAGCAAAAATAATCCCAAAGC (N) 5′-GCTTTGGGATTATTTTTGCTAAACCCATGCACAAATTAGGTGATCATG	
Introduce mutated RAP1 binding site into *HIS7* promoter	(O) 5′-CATGATCACCTAATTTGTGCATGGGTTTAGCAAAAATAATCCCAAAGC (P) 5′-GCTTTGGGATTATTTTTGCTAAACCCATGCACAAATTAGGTGATCATG	
Introduce mutated ABF1 binding site into *HIS7* promoter	(Q) 5′-CATGATCACCTAATTGTCACTACCACCGAAAAATAATCCCAAAGC (R) 5′-GCTTTGGGATTATTTTTCGGTGGTAGTGACAATTAGGTGATCATG	
Introduce SacI site into *ADE5,7* promoter	(S) 5′-GATCTAGAGCTCTGTTACACGCAGCATCGTTCTTTGG	SacI
Introduce BamHI site into *ADE5,7* promoter	(T) 5′-GCTATGGATCCTGTGAGGGGAGGGAGAATGGTTCTC	BamHI
Introduce RAP1 binding site into *ADE5,7* promoter	(U) 5′-GTCAGTCGGCACTTTGTGCATGGGTTTAGCGGGCGAGTCAACTG (V) 5′-CAGTTGACTCGCCCGCTAAACCCATGCACAAAGTGCCGACTGAC	
Introduce mutated RAP1 binding site into *ADE5,7* promoter	(W) 5′-GTCAGTCGGCACTTTGTGCATGGGTTTAGCGGGCGAGTCAACTG (X) 5′-CAGTTGACTCGCCCGCTAAACCCATGCACAAAGTGCCGACTGAC	
Introduce mutated ABF1 binding site into *ADE5,7* promoter	(Y) 5′-GTCAGTCGGCACTTGTCACTACCACCGGGGGCGAGTCAACTG (Z) 5′-CAGTTGACTCGCCCCGGTGGTAGTGACAAGTGCCGACTGAC	
ACT1	(1) 5′-CCATGCCTAGACAAATCAAGGAAAGTATGTC (2) 5′-GGAGAGAGAGAGGCGAGTTTGGTTTCAAAACG	
SPT15	(3) 5′-CCCCTCTGATAGCTGAGATGTCGGGATTCC (4) 5′-CAGTAACTACTGTAATTTTCACGTCCCTTG	
HIS4	(5) 5′-GGATATGACTATGAACAGTAGTATACTGTG (6) 5′-TCCCAACCCATCTGTGGAGTGAG	

episomes designed to place mutant or wild-type RAP1 or ABF1 binding sites into a positioned nucleosome (Fig. 1A). These plasmids are based on the yeast *TRP1ARS1* plasmid, which is packaged into stably positioned nucleosomes in yeast (52). The chromatin structures of TA/GCN1Δ80, which has a GCN4 binding site in nucleosome I, and TAR/GCN1Δ80, which has a RAP1 binding site adjacent to the GCN4 binding site, have been documented in previous studies (58). TAA/GCN1Δ80 is identical to TAR/GCN1Δ80, except that an ABF1 binding site replaces the RAP1 binding site in nucleosome I.

Since *ABF1* is an essential gene (39), we introduced a mutated ABF1 site (40) into nucleosome I as a control. These four episomes were introduced into yeast, and nucleosome positioning was examined by indirect end labeling. This was done in GCN4+ yeast cells, as we have found that the chromatin structure of TA/GCN1Δ80, TAR/GCN1Δ80, and TARmut/GCN1Δ80 is not affected significantly by the presence or absence of GCN4 (data not shown). In the indirect end-labeling assay, MNase cleavage sites are compared in naked DNA and chromatin, and regions of 140 to 160 bp that are protected in

TABLE 2. Yeast strains used in this study

Strain	Genotype or description	Reference or source
FY24	*MATα ura3-52 trp1Δ63 leu2Δ1*	57
LYY50	Same as FY24 but *gcn4Δ*	58
YDS2	*MATa ade2-1 can1-100 his3-11,15 leu2-3,112 trp1-1 ura3-1*	18
YDS408	*MATa ade2-1 can1-100 his3-11,15 leu2-3,112 trp1-1 ura3-1 rap1-2ts*	18
TMY86	*mata ade2-1 his3-11,15 leu2-3,112 trp1-1 ura3-1 can1-100 abf1Δ::HIS3MX6* [pRS416-*ABF1*]	26
1-731	*TMY86-1/pRS415-ABF1-1-731*	26
1-662	*TMY86-1/pTM211; pRS415-ABF1-1-662*	26
1-633	*TMY86-1/pTM487; pRS415-ABF1-1-633*	26
1-592	*TMY86-1/pTM629; pRS415-ABF1-1-592*	26
abf1 ts strain	*TMY86-1/pTM629; pRS415-abf1-1*	26
YS18	*MATα his3-11 his3-15 leu2-3,112 ura3Δ5 canR*	1
YS19	*MATα his3-11 his3-15 leu2-3,112 ura3Δ5 canR bas2⁻*	1
MSY202	*MATα ura3-52 lys2Δ201 leu2-3,112 trp1-289 HHT1-HHF1 Δ(HHT2-HHF2)*	43
AY(1-731)	Same as 1-731 but *gcn4Δ*	Present study
AY(1-592)	Same as 1-592 but *gcn4Δ*	Present study

chromatin, but not in naked DNA, are diagnostic of positioned nucleosomes (46, 51).

Nucleosomes I and II were positioned in TAAmut /GCN1Δ80 as in TA/GCN1Δ80 (compare Fig. 1B, lanes 5 to 7, and 1C, lanes 4 to 8). The features of the MNase cleavage pattern diagnostic of this positioning are the two cleavage sites that are protected in chromatin relative to naked DNA (asterisk and open circle, Fig. 1B, lanes 2 to 7, and Fig. 1C, lanes 1 to 8) and the three cleavage sites enhanced in chromatin relative to naked DNA. The latter sites are separated by about 150 bp and mark the edges of positioned nucleosomes (closed circles) (28, 30, 52, 58). In contrast, the chromatin structure of TAR/GCN1Δ80 and TAA/GCN1Δ80 dramatically changed. As previously reported, the MNase cleavage pattern for TAR/ GCN1Δ80 chromatin is essentially identical to that for the corresponding naked DNA in the regions of nucleosomes I and II, indicating that nucleosome positioning in this region is abolished (Fig. 1B, lanes 8 to 14) (58). The chromatin structure of TAA/GCN1Δ80 is similar to that of TAR/GCN1Δ80 but not identical (compare Fig. 1B, lanes 13 and 14, to Fig. 1C, lanes 13 to 15). Both TAA/GCN1Δ80 and TAR/GCN1Δ80 chromatin show MNase cleavage in the region of nucleosome I, at a site which is cleaved weakly in naked DNA but is completely protected in TA/GCN1Δ80 and in the mutABF1 plasmid chromatin (open circle in Fig. 1B and C). At the border between nucleosomes I and II, cleavage by MNase is enhanced in TA/ GCN1Δ80 and in the mutABF1 plasmids relative to that in naked DNA (middle closed circle, Fig. 1B, lanes 2 to 8, and Fig. 1C, lanes 1 to 8) but is not enhanced relative to the naked DNA pattern in TAR/GCN1Δ80 (Fig. 1B, lanes 8 to 14). In TAA/GCN1Δ80, this cleavage site appears slightly protected relative to naked DNA, possibly indicating some nucleosomal protection (Fig. 1C, lanes 10 to 15). The cleavage site that is largely protected by nucleosome II in TA/GCN1Δ80 and TAAmut/GCN1Δ80 chromatin (Fig. 1B, lanes 2 to 7, and Fig. 1C, lanes 1 to 8) is not at all protected in TAR/GCN1Δ80 (Fig. 1B, lanes 8 to 14) but still shows some protection in TAA/ GCN1Δ80 (Fig. 1C, lanes 2 to 7). Similarly, the enhanced cleavage site at the distal border of nucleosome II in TA/ GCN1Δ80 and TAAmut/GCN1Δ80 (uppermost closed circle, Fig. 1B and C) is not at all enhanced in TAR/GCN1Δ80 but

still shows some enhanced cleavage in TAA/GCN1Δ80. Taken together, these results demonstrate that ABF1, like RAP1, is effective in creating a localized region of open chromatin. However, whereas RAP1 abolishes positioning of two nucleosomes, ABF1 appears to clear the nucleosome in the immediate vicinity of its binding site but still allows positioning, or partial positioning, nearby (Fig. 1D). These results indicate that ABF1 may be able to function similarly to RAP1 in opening chromatin to allow transactivator access and thus facilitate transcription, although it may differ subtly in the mechanism by which it does so.

Contribution of C-terminal domains to chromatin perturbation by ABF1. Having established that ABF1 shares with RAP1 an ability to perturb nucleosome positioning via a nucleosomal binding site in vivo (Fig. 1), we next wanted to test whether domains of ABF1 outside the DNA-binding domain were important for this property. Both RAP1 and ABF1 possess central DNA-binding domains and putative transactivation domains in their C-terminal regions (Fig. 2) (13, 20, 26). Furthermore, RAP1 and ABF1 share a short region of homology in their putative transactivation domains (5, 26). Somewhat surprisingly, we have found that the putative transactivation domain, and indeed most of the C-terminal region of RAP1, is dispensable for chromatin perturbation via a nucleosomal binding site as well as for activation of *HIS4* (59). In contrast, the C-terminal region of ABF1 has been shown to contain two domains, CS1 and CS2, that contribute to transcriptional activation as well as to chromatin remodeling (but from binding sites located nearby and not within the remodeled nucleosomes) (26). To test the involvement of the C-terminal region of ABF1 in chromatin perturbation via a nucleosomal binding site, we tested the abilities of truncated C-terminal derivatives of ABF1 to perturb the chromatin structure of TAA/GCN1Δ80 relative to TAAmut/GCN1Δ80 in yeast.

Figure 3A shows the results of indirect end-label analysis following MNase digestion of the episomes TAA/GCN1Δ80 and TAAmut/GCN1Δ80, which contain a wild-type or mutant ABF1 site near the center of positioned nucleosome I of TA/ GCN1Δ80 (Fig. 1), in yeast cells in which the chromosomal *ABF1* gene has been deleted and which express full-length ABF1(1-731) from a *CEN* plasmid. The MNase digestion pat-
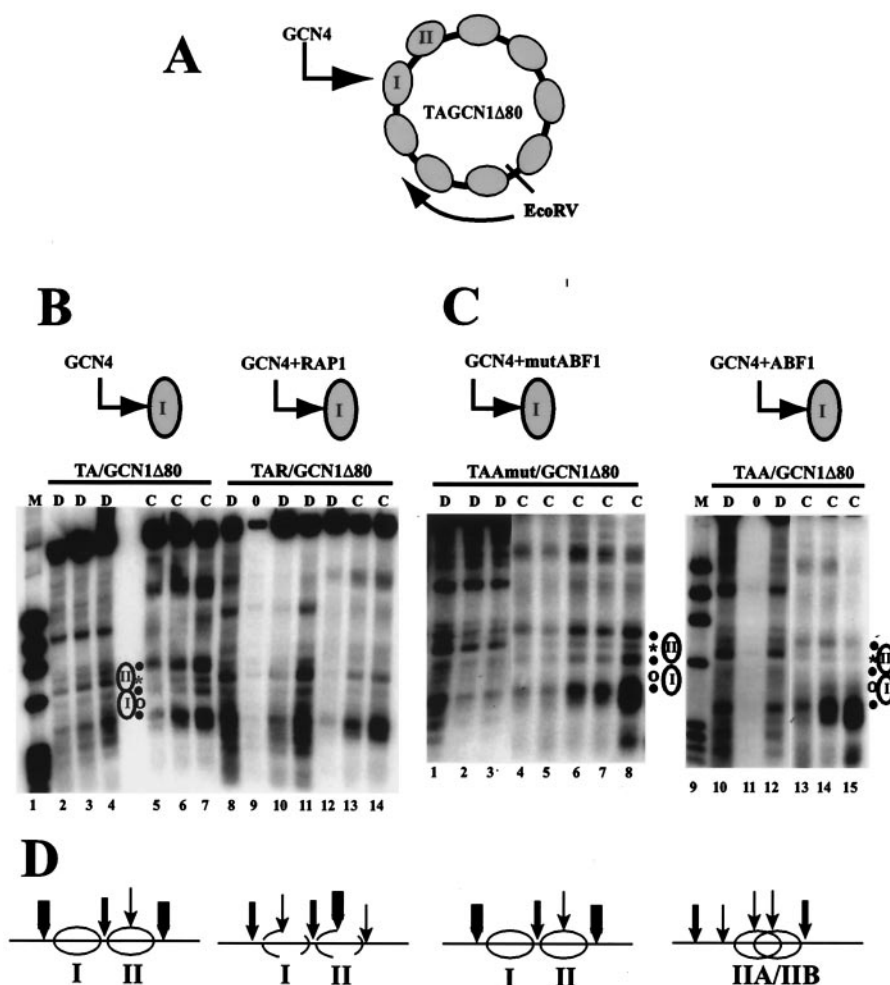
FIG. 1. ABF1 perturbs nucleosome positioning via a nucleosomal binding site similarly to RAP1. (A) Schematic diagram of plasmid TA/GCN1Δ80. The gray ellipses represent positioned nucleosomes, and nucleosomes I and II are marked. Nucleosome I contains the GCN4 binding site, as shown, and chromatin structure was mapped clockwise from the EcoRV site, as indicated, by use of chromatin prepared from FY24 yeast cells containing the indicated plasmid episome. (B) Indirect end-label analysis of TA/GCN1Δ80 and TAR/GCN1Δ80 chromatin structure. TAR/GCN1Δ80 is similar to TA/GCN1Δ80 except for a RAP1 binding site introduced adjacent to the GCN4 binding site. MNase cleavage sites were mapped clockwise from the EcoRV site in naked DNA (D lanes) or in chromatin (C lanes) from cells grown in glucose medium under conditions that are noninducing for Gcn4p activation. Lane 1 contains φX/HaeIII marker DNA. Naked DNA in TA/GCN1Δ80 (lanes 2 to 4) and in TAR/GCN1Δ80 (lanes 8, 10, and 11) was digested using 2.5 (lanes 2 and 8), 5 (lanes 3 and 10), or 10 (lane 4 and 11) U of MNase per ml. Chromatin was digested using 0 (lane 9), 2.5 (lanes 5 and 12), 5 (lanes 6 and 13), or 10 (lanes 7 and 14) U/ml. (C) Indirect end-label analysis of TAA/GCN1Δ80 and TAAmut/GCN1Δ80 chromatin structure. TAA/GCN1Δ80 and TAAmut/GCN1Δ80 are similar to TA/GCN1Δ80, except for ABF1 or mutated ABF1 binding sites introduced adjacent to the GCN4 binding site. MNase cleavage sites were mapped clockwise from the EcoRV site in naked DNA (D lanes) or in chromatin (C lanes) from cells grown in glucose medium. Lane 9 contains φX/HaeIII marker DNA. Naked TAA/GCN1Δ80 and TAAmut/GCN1Δ80 DNA were digested using 2.5 (lanes 1 and 10), 5 (lanes 2 and 12), or 10 (lane 3) U/ml. Chromatin was digested using 0 (lane 11), 2.5 (lanes 4 and 13), 5 (lanes 5 and 14), 10 (lanes 6 and 15), 15 (lane 7), or 20 (lane 8) U of MNase per ml. All the chromatin samples were run on the same gel; the DNA lanes are derived from separate gels (Fig. 3 shows examples in which chromatin and naked DNA samples all derive from the same gel). The asterisks and open circles indicate cleavages in naked DNA that are protected by nucleosomes I and II in chromatin, and filled circles indicate the edges of nucleosomes I and II, which are present in TA/GCN1Δ80 and TAAmut/GCN1Δ80 but not in TAR/GCN1Δ80 and TAA/GCN1Δ80. The locations of positioned nucleosomes I and II are shown by ellipses. (D) Schematic diagram of nucleosome positioning in the region of nucleosomes I and II deduced from the MNase cutting patterns of TA/GCN1Δ80, TAR/GCN1Δ80, TAA/GCN1Δ80, and TAAmut/GCN1Δ80. The thickness of each vertical arrow indicates the relative strength of MNase cleavage.

tern of TAA/GCN1 chromatin is essentially identical to that seen in Fig. 1, in which ABF1 was expressed from its normal chromosomal location. Cleavage is seen in TAA/GCN1Δ80 in the region of nucleosome I that is protected in TAAmut/GCN1Δ80 (compare lanes 6 to 8 and lanes 13 to 15 in Fig. 3A; band marked by asterisk), and the cleavage site between nu-

cleosomes I and II in TAAmut/GCN1Δ80 is mostly protected in TAA/GCN1Δ80 (central closed circle in Fig. 3A). Thus, as seen in Fig. 1, full-length ABF1 evidently has a strong ability to perturb nucleosome positioning in vivo via a nucleosomal ABF1 binding site. The same MNase cleavage pattern was obtained using yeast expressing ABF1(1-662) (Fig. 3B). This

A

DNA binding domains

Transactivation domain

ABF1 1-731

1 731

CS1 CS2

B

ABF1 1-662

1 662

CS1 CS2

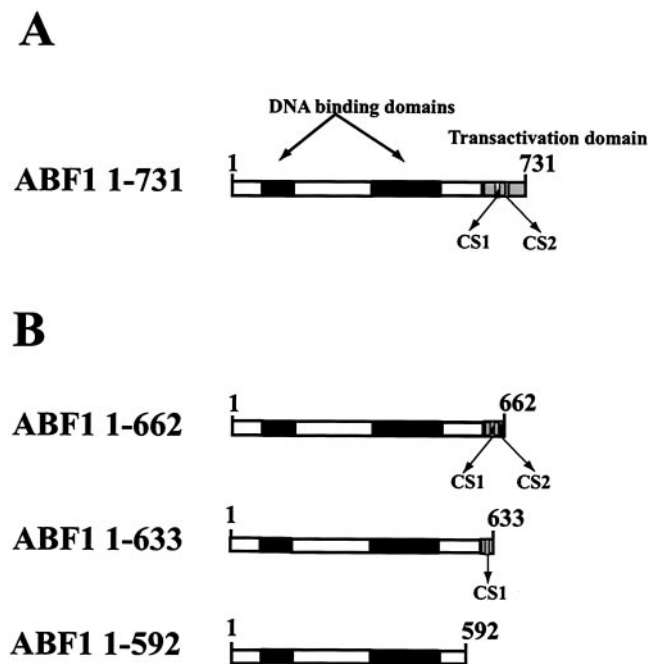ABF1 1-633

1 633

CS1

ABF1 1-592

1 592

FIG. 2. Schematic diagram of ABF1. (A) Full-length ABF1 is shown, with DNA-binding domains, transactivation domain, and the C-terminal sequences (CS1 and CS2) indicated. (B) The truncated versions of ABF1 used in this work are shown.

was not surprising, as both CS1 and CS2 are still present in this construct, and no growth defect was observed in this yeast strain (26). Further deletion of ABF1 to remove the CS2 domain [ABF1(1-633)] leads to growth defects at 30 and 35°C and a weak *ts* phenotype at 37°C and also abolishes the ability of ABF1 to remodel a nucleosome from a nearby site in vivo (26). The ability of ABF1(1-633) to perturb TAA/GCN1Δ80 chromatin was, however, undiminished compared to that of wild-type ABF1 (Fig. 3C). Further deletion to remove both CS1 and CS2 domains [ABF1(1-592)] still did not impair the ability of ABF1 to perturb TAA/GCN1Δ80 nucleosome positioning, with cleavage in the region of nucleosome I and partial protection of the cleavage site between nucleosomes I and II being readily apparent (Fig. 3D). We conclude that, although CS1 and CS2 domains of ABF1 are needed to remodel chromatin from sites near a positioned nucleosome, they are not needed to outcompete histones for binding to the same sequence with concomitant perturbation of nucleosome positioning. This result also demonstrates that chromatin can be remodeled by more than one mechanism in vivo.

ABF1 can contribute to activation of *HIS4* similarly to RAP1. Previous work has provided strong evidence that RAP1 functions at the *HIS4* promoter at least in part by opening chromatin to allow binding by the primary activators, GCN4 and Bas2p/Bas2p (4, 58). Correspondingly, RAP1 is very effective in outcompeting histones for binding to a nucleosome positioning sequence in yeast (58). Since ABF1 shares this latter property with RAP1 (Fig. 1 and 3), we wanted to test whether ABF1 could substitute for RAP1 to help GCN4 activate the *HIS4* promoter. A *MEL1* reporter gene, encoding α-galactosidase, was fused to the wild-type *HIS4* promoter, or

to the *HIS4* promoter in which the RAP1 site has been mutated, and also to a modified *HIS4* promoter having an ABF1 binding site or mutated ABF1 binding site adjacent to the GCN4 binding site. α-Galactosidase activity was monitored and compared between GCN4+ wild-type cells and *gcn4Δ* cells for all four promoters. The results shown in Fig. 4A demonstrate that, for the native *HIS4* promoter, mutation of the RAP1 binding site or absence of GCN4 results in substantial loss of transcription, as expected (4). This synergism between RAP1 and GCN4 is consistent with RAP1 opening chromatin to facilitate access by GCN4, the principal activator. Replacement of the RAP1 binding site by a binding site for ABF1, or a mutated binding site, resulted in similar behavior (Fig. 4A). We do not know why the *HIS4* promoter possessing a mutated ABF1 site has higher activity than that of the promoter with the mutated RAP1 site, as previous work indicated that ABF1 showed essentially no activity from this mutated site (40). In any event, these results are consistent with the notion that ABF1 shares with RAP1 an ability to facilitate GCN4 binding to the *HIS4* promoter by overcoming the repressive effect of chromatin.

The C-terminal portion of ABF1, and in particular the short CS2 domain, is evidently not required for perturbation of chromatin via a nucleosomal ABF1 binding site (Fig. 3). However, this domain is required for chromatin remodeling from sites near positioned nucleosomes and is also important for transcriptional activation (26). Thus, depending on the mechanism by which ABF1 perturbs chromatin at the modified *HIS4* promoter, the C-terminal region and CS2 domain may or may not be required to facilitate *HIS4* transcriptional activation. To examine this question, we introduced the modified *HIS4-MEL1* reporter into cells expressing full-length or truncated ABF1 proteins, as in Fig. 3, and measured α-galactosidase activity. The results, shown in Fig. 4B, indicate that loss of amino acids 663 to 731 from ABF1 results in only a slight decrease in *HIS4* activity. Loss of CS2 (amino acids 639 to 662) results in diminished transcriptional activity ($P < 0.003$ by Student *t* test), and loss of CS1 lowers activity even further ($P < 0.001$). However, ABF1 increases *HIS4* activation by GCN4 even when both CS1 and CS2 are absent [ABF1(1-592)], although it can no longer stimulate transcription of *HIS4* in the absence of GCN4 (Fig. 4C). This strongly suggests that ABF1 stimulates transcription from the modified *HIS4* promoter in part through its CS1 and CS2 domains but also in part by remodeling chromatin in the immediate vicinity of its binding site via a mechanism independent of CS1 and CS2.

Comparison of ABF1 and RAP1 in facilitating transcriptional activation from the *HIS7* and *ADE5,7* promoters. We next examined two additional promoters containing ABF1 binding sites, *HIS7* and *ADE5,7*. The *HIS7* promoter contains an ABF1 binding site approximately midway between two GCN4 binding sites at −231/−225 and −144/−139 bp relative to the translational start codon, and the region surrounding the ABF1 binding site and including both GCN4 binding sites has recently been shown to be nucleosome free (47, 53). In addition, Bas1p/Bas2p can activate *HIS7* via the −144/−139 site (48). The ABF1 binding site was found to be essential for basal transcription of a *HIS7-lacZ* fusion gene in *gcn4−* yeast (47). We examined GCN4-dependent activation of a *HIS7-MEL1* reporter gene and found modest stimulation by either ABF1 or
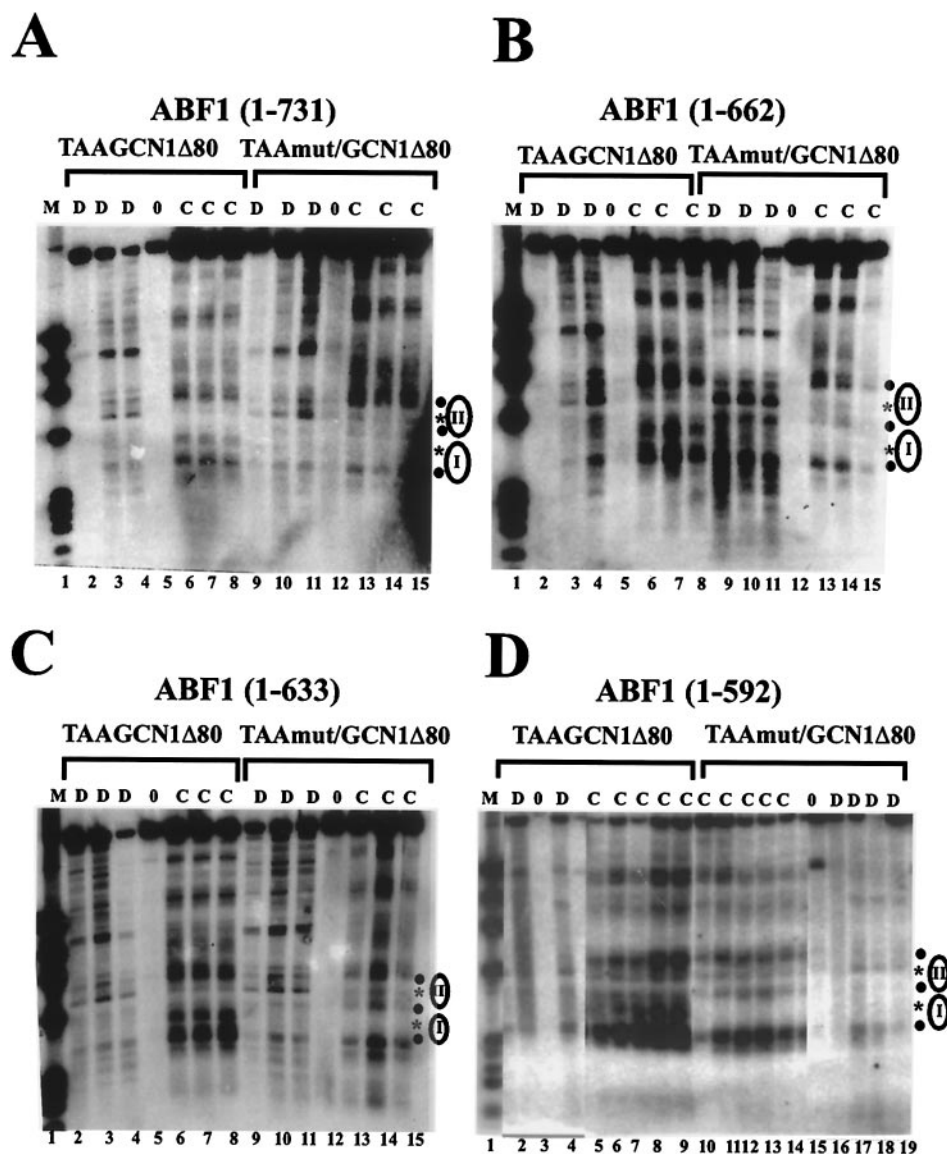
FIG. 3. Contribution of C-terminal domains of ABF1 to chromatin perturbation via a nucleosomal binding site. (A) MNase cleavage sites in plasmids TAA/GCN1Δ80 and TAAmut/GCN1Δ80 were mapped in GCN4^{+} yeast harboring ABF1(1-731). Cleavage sites were mapped in naked DNA (D lanes) or in chromatin (C lanes) from the EcoRV site, as in Fig. 1. Lane 1 contains φX/HaeIII marker DNA. Locations of positioned nucleosomes I and II are indicated by ellipses. The filled circles to the right of the panel indicate cleavages enhanced in chromatin relative to DNA, and the stars indicate strong cleavages in naked DNA that are protected by nucleosomes I and II in chromatin of TAAmut/GCN1Δ80 but not TAA/GCN1Δ80. Each lane, beginning with lanes 2 to 4, differs only in the concentration of MNase used. Lanes 5 and 12 show controls not treated with MNase. (B) MNase cleavage sites in plasmids TAA/GCN1Δ80 and TAAmut/GCN1Δ80 were mapped in yeast harboring ABF1(1-662), as in panel A. (C) MNase cleavage sites in plasmids TAA/GCN1Δ80 and TAAmut/GCN1Δ80 were mapped in yeast harboring ABF1(1-633) as in panel A. (D) MNase cleavage sites in plasmids TAA/GCN1Δ80 and TAAmut/GCN1Δ80 were mapped in yeast harboring ABF1(1-592) as in panel A. All samples were run on the same gel, but some lanes are omitted from the figure, as seen from the visible "splice" marks.

GCN4 alone (Fig. 5A). Activation with the wild-type *HIS7* promoter in GCN4^{+} yeast was about twofold stronger than the summed individual effects of GCN4 and ABF1 (Fig. 5A). Substitution of a RAP1 or mutated RAP1 binding site for the ABF1 binding site in the *HIS7* promoter yielded similar results, although the synergism between RAP1 and GCN4 was somewhat less than that between ABF1 and GCN4 (Fig. 5A). We conclude that ABF1 and RAP1 can function interchangeably at the *HIS7* promoter, with each showing modest (1.5- to

2-fold) synergism with GCN4. This suggests that ABF1 can facilitate GCN4-mediated activation, possibly by opening chromatin, in the context of a natural promoter and that RAP1 can provide this same function at a suitably modified promoter.

The *HIS4* promoter can be activated by either GCN4 or, via an alternative pathway, BAS1/BAS2. In either case, the RAP1 binding site, which is located between binding sites for BAS1 and GCN4, is needed to facilitate activation (4). The *ADE5,7* promoter is activated by BAS1/BAS2 in response to purine
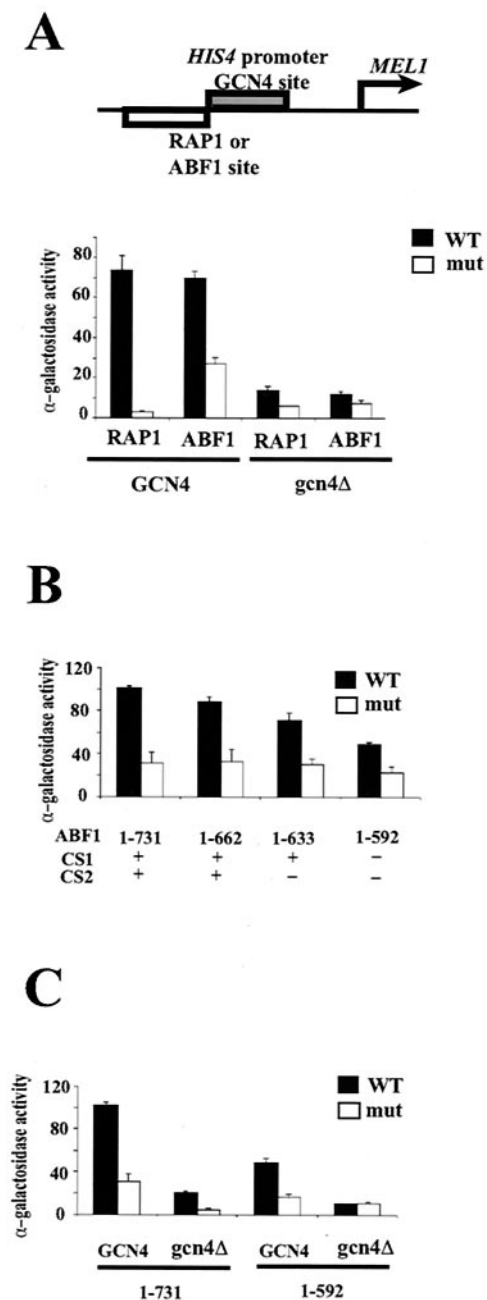
FIG. 4. ABF1 can contribute to activation of the *HIS4* promoter similarly to RAP1. (A) The *MEL1* reporter gene fused to modified *HIS4* promoter having an ABF1 or RAP1 binding site or mutant ABF1 or RAP1 binding sites adjacent to the GCN4 binding site, schematized at the top. *MEL1* activity was monitored in GCN4+ cells or *gcn4*Δ cells and expressed as α-galactosidase activity. (B) Contribution of C-terminal domains to GCN4-mediated activation of the *HIS4* promoter. The modified *HIS4* promoter having an ABF1 binding site or mutant ABF1 binding site adjacent to the GCN4 binding site was introduced into yeast expressing ABF1 deletion mutants as indicated, and *MEL1* activity was monitored. (C) *MEL1* activity was monitored from the modified *HIS4* promoter having an ABF1 binding site or mutant ABF1 binding site adjacent to the GCN4 binding site in GCN4+ or *gcn4*Δ yeast harboring full-length ABF1 ("1-731") or truncated ABF1 lacking the CS1 and CS2 domains ("1-592"), as indicated. Standard deviations are indicated. WT, wild type.

starvation, and an ABF1 binding site about 20 bp upstream of the proximal and more important of the two BAS1 binding sites is essential for this activation (40). If RAP1 and ABF1 both facilitate activation in part by opening chromatin, we would expect the two proteins to be able to assist different activators (as RAP1 can do at the *HIS4* promoter) (4, 58). We therefore tested whether RAP1 could substitute for ABF1 to facilitate BAS1/BAS2-mediated activation of the *ADE5,7* promoter. A *MEL1* reporter gene was fused to the wild-type and modified versions of the *ADE5,7* promoter having wild-type or mutant ABF1 or RAP1 binding sites (Fig. 5B). Both BAS1 and BAS2 are required for activation in response to adenine depletion (40); we found that, in the absence of BAS2, activation by RAP1 or ABF1 is very modest and independent of adenine depletion (Fig. 5B). In BAS2+ yeast, activation in response to adenine depletion depends strongly on the presence of a binding site for ABF1 or RAP1, with the two sites providing similar levels of stimulation (Fig. 5B). Thus, at three distinct yeast promoters, ABF1 and RAP1 can each synergize with GCN4, with BAS1/BAS2, or with both to activate transcription, most likely by opening chromatin to facilitate binding by the principal activator.

Continuous ABF1 binding to DNA is not required to maintain GCN4-mediated activation facilitated by ABF1. Previous results have shown that continuous RAP1 binding is required for ongoing *HIS4* transcription mediated by GCN4, even in nonreplicating cells (59). In contrast, several "ABF1-driven" genes (*SPT15*, *RPL2A*, *RPL2B*, *TCM*, and *QCR8*) apparently do not require continuous ABF1 occupancy of the ABF1 *cis* element for continuous high-level transcription (44). This could reflect differences in the mechanisms by which ABF1 and RAP1 contribute to transcriptional activation, or it could reflect differences between the *HIS4* promoter and the ABF1-dependent promoters examined previously. To directly compare the requirements for continuous binding of RAP1 and ABF1 to stimulate transcriptional activation, we therefore examined whether continuous ABF1 binding is required for efficient GCN4-mediated activation of the modified *HIS4* promoter in which an ABF1 site replaces the RAP1 site.

In this experiment, we used an *abf1 ts* mutant that is temperature sensitive for binding to DNA in vivo and in vitro (38, 44). The *abf1-1* mutant encodes a mutation in the putative zinc finger motif in the DNA-binding domain of ABF1. Dimethyl sulfate footprinting has shown that this mutant ABF1 protein vacates its binding site in vivo within 1 h, and at some promoters within 3 to 4 min, following a shift to the nonpermissive temperature of 37°C (44). We examined *MEL1* mRNA levels expressed from the normal and modified *HIS4* promoters before and after shifting the cells from permissive temperature to the nonpermissive temperature. Cells were grown at 25°C overnight to log phase and were then inoculated into fresh medium preequilibrated to the appropriate temperature and grown at either 25 or 37°C. We then harvested mRNA from cells at various time points. We chose our first time point for mRNA sampling at 1 h after the temperature shift to allow ample time for the decay of preexisting *MEL1* mRNA. As shown in Fig. 6A, the expression of *MEL1* mRNA from the modified *HIS4* promoter containing an ABF1 binding site in *abf1-1ts* cells was unchanged at 25 and 37°C. In contrast, levels of *MEL1* mRNA expressed from the normal *HIS4* promoter
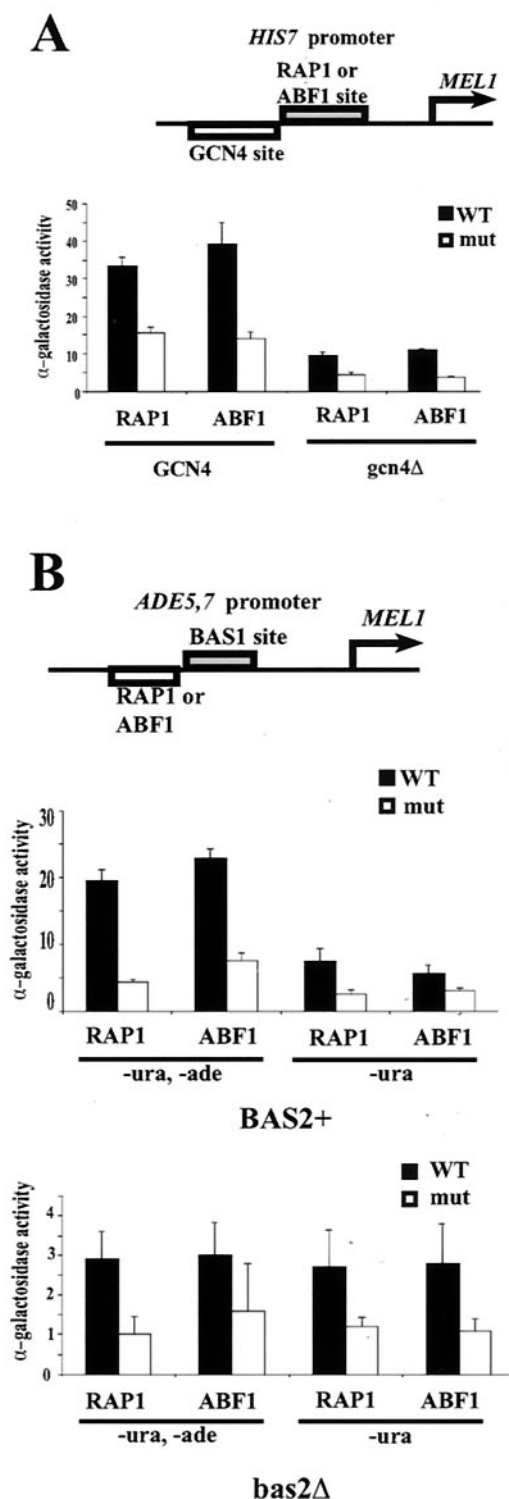
FIG. 5. Comparison of ABF1 and RAP1 in facilitating transcriptional activation from the *HIS7* and *ADE5,7* promoters. (A) The *MEL1* reporter gene fused to a modified *HIS7* promoter having an ABF1 binding site or RAP1 binding site or mutant ABF1 or RAP1 site adjacent to the GCN4 binding site, schematized at the top. *MEL1* activity was monitored and compared in GCN4^{+} wild-type cells and in *gcn4Δ* cells. Standard deviations are shown. (B) The *MEL1* reporter gene fused to a modified *ADE5,7* promoter having an ABF1 binding site or RAP1 binding site or mutant ABF1 or RAP1 binding site adjacent to the BAS1 binding site, schematized at the top. *MEL1* activity was monitored and compared in BAS1 and BAS2^{+} wild-type cells and in *bas2Δ* cells (note different scales) in the presence and absence of adenine. Standard deviations are shown. WT, wild type.

containing a RAP1 binding site declined substantially relative to that of a *PYK1* control at 37°C in the *rap1-2[ts]* strain, consistent with previous results (59) (Fig. 6B). (We also showed previously that *HIS3* mRNA, which has a shorter half-life than does the *PYK1* message [23 min compared to about 60 min (55)], was similarly unaffected by the temperature shift in this strain [59].) Similar results were observed in four independent experiments. Furthermore, the results in Fig. 6C show that the level of *MEL1* mRNA produced from the template having a mutant ABF1 site is reduced about fourfold relative to that of the template having a wild-type ABF1 site in yeast grown at 37°C, and a similar decrease was observed at 25°C (data not shown). Thus, if loss of ABF1 binding at 37°C in the *ts* mutant in the experiment whose results are shown in Fig. 6A led to a decrease in *MEL1* mRNA levels corresponding to those produced from the template having a mutant ABF1 binding site (Fig. 6C), this decrease would be readily observable.

To test the possibility that ABF1 remains bound to the modified *HIS4* promoter after a shift to the nonpermissive temperature, we performed ChIP using antibodies to ABF1. To visualize only the modified, plasmid-borne *HIS4* promoter, we used one primer from the *HIS4* promoter and another from the *MEL1* coding sequence in our analysis. The results in Fig. 7A show that immunoprecipitation with the use of antibodies to ABF1 substantially enriched the modified *HIS4* promoter compared to an *ACT1* control, as expected. (The *ACT1* product was amplified considerably more efficiently than was the modified *HIS4* promoter or the *SPT15* promoter [Fig. 7B], for reasons that we do not understand.) In cells expressing full-length, wild-type ABF1, no change is seen upon shifting the temperature to 37°C (Fig. 7A, lanes 1 and 2, and 7C). In contrast, shifting *abf1-1 ts* cells to 37°C resulted in about a twofold loss of IP *HIS4* fragment compared to that for 25°C. The level of IP fragment relative to input seen after the temperature shift was comparable to that seen with a template having the mutant ABF1 binding site (data not shown), indicating that it represents nonspecific background. Quantitatively similar results were seen with the *SPT15* promoter (Fig. 7B and C), indicating that loss of ABF1 at the restrictive temperature in the *abf1-1 ts* mutant is similar at the two promoters. Since loss of ABF1 from the *SPT15* promoter was shown to be complete under these conditions by dimethyl sulfate footprinting (44), we conclude that complete or nearly complete loss of ABF1 also likely occurred from the modified *HIS4* promoter at 37°C in the *ts* mutant. Thus, the requirement for continuous binding of RAP1, but not ABF1, to facilitate activated transcription reflects a fundamental mechanistic difference between these two otherwise functionally similar GRFs, rather than a difference between individual promoters either requiring or not requiring continuous GRF occupancy.

DISCUSSION

ABF1 and RAP1 are multifunctional proteins that are grouped together with GRF2/REB1 as GRFs (6). The func-
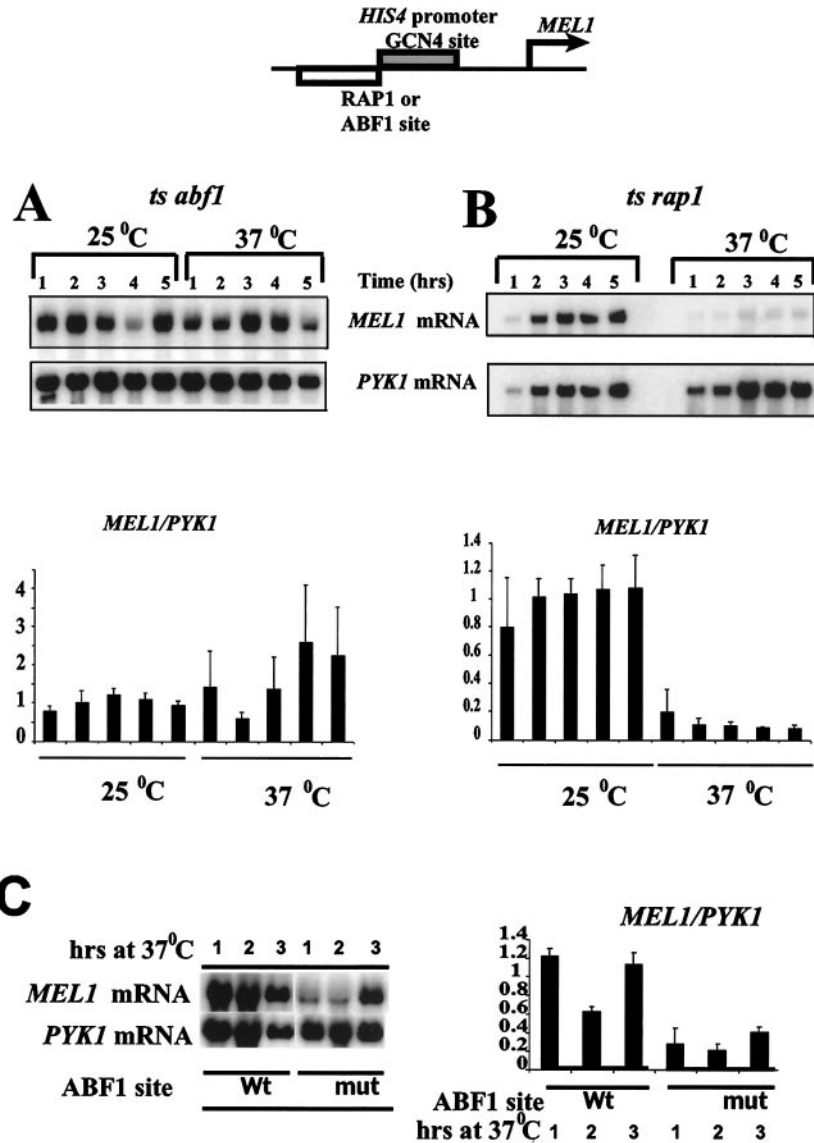
FIG. 6. Continuous ABF1 binding to DNA is not required for efficient GCN4-mediated *HIS4* activation. RNA was harvested from *abf1 ts* (A) and *rap1-2 ts* (B) cells harboring the *HIS4-MEL1* reporter (schematized at the top) with either an ABF1 binding site (A) or a RAP1 binding site (B) after growth for the indicated times, in hours, at either 25 or 37°C. The RNA blots were hybridized with probes specific for *MEL1* and *PYK1* mRNA, and the signals were quantitated by PhosphorImager analysis. The bar graphs at the bottom show values derived from four independent experiments. Values for each experiment were normalized to the average *MEL1/PYK1* value obtained for all time points at 25°C, and standard deviations are indicated. (C) Comparison of *MEL1* mRNA levels (normalized to those of *PYK1*) from the modified *HIS4* promoter having a wild-type or mutant ABF1 site, as indicated, after 1 to 3 h of growth at 37°C. All six lanes (for each mRNA) derive from a single exposure of the same blot. Quantitations in the bar graph on the right are from three independent experiments and were scaled by setting the average *MEL1-PYK1* value for all three time points for the promoter having the wild-type ABF1 site to 1.

tional similarities of ABF1 and RAP1 have been appreciated for some time, and the two factors have been compared with respect to their abilities to activate transcription from ribosomal protein gene promoters, to stimulate replication origin activity, to support cell viability, and to function as barriers between euchromatin and heterochromatin (6, 8, 9, 22, 24, 37, 60). However, other relevant properties of ABF1 and RAP1 have not been directly compared in previous work. First, although both ABF1 and RAP1 can perturb or remodel chro-matin structure (2, 16, 54, 58), this function has not previously been compared for the same chromatin template. Second, the ability to open chromatin likely contributes to both ABF1 and RAP1 function at some promoters (3, 4, 16, 19, 23, 29, 40, 58), but the interchangeability of ABF1 and RAP1 binding sites at such promoters has not been previously examined. Third, although both ABF1 and RAP1 have carboxy-terminal regions with multiple important functions, the contributions of these regions to particular processes have not been directly com-
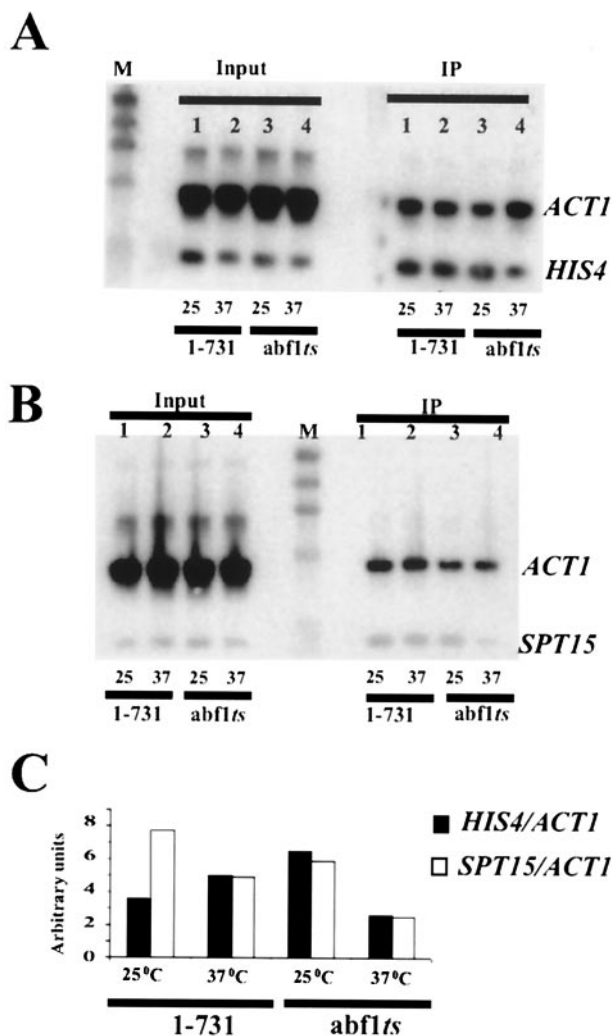
FIG. 7. ChIP analysis of ABF1 binding to the modified *HIS4-MEL1* promoter. ChIP was performed with ABF1 antibody in yeast carrying ABF1(1-731) or the *abf1-1 ts* mutant at 25°C or after 1 h at 37°C, as indicated. Input and IP samples were amplified in separate reactions with the use of primers for the *HIS4-MEL1*, *SPT15*, and *ACT1* promoters. Samples were combined prior to electrophoresis, Southern blotting, and hybridization with appropriate probes. Although *ACT1* was used as a negative control, its amplification was more efficient than that of *HIS4-MEL1* or *SPT15* promoters, for unknown reasons. Note, however, that *ACT1* shows reduction in the IP lanes relative to the input lanes, and *HIS4-MEL1* and *SPT15* do not. Ratios of *HIS4-MEL1* IP samples to input samples and *SPT15* IP samples to input samples, relative to the ratio of *ACT1* IP samples to input samples, are shown at the bottom. Similar results were obtained in this and one other independent experiment.

pared. Fourth, ABF1 has been shown not to be continuously required at several ABF1-driven promoters for continued transcription, whereas RAP1 is needed for ongoing GCN4-mediated *HIS4* activation (44, 59), but a comparison of this property at a single promoter was lacking.

In this paper, we have compared ABF1 and RAP1 in the functional assays delineated above. Previous work had shown that ABF1 could create a region of open chromatin in its immediate vicinity (although a direct test of its ability to out-

compete histones for its binding site had not been performed) (16, 19, 26). We were therefore not surprised to find that ABF1, like RAP1, can perturb chromatin structure when its binding site is incorporated into the region of a positioned nucleosome (Fig. 1). However, although the C-terminal region is required for ABF1 to remodel nucleosomes from a nearby, accessible binding site (26), it is not required for chromatin perturbation via a nucleosomal binding site (Fig. 3). Similarly, RAP1 can perturb chromatin via a nucleosomal binding site independently of its C-terminal region (59). Previous work has indicated that DNA-binding proteins can compete with histones for the same binding site by mass action in vitro and in vivo (25, 34, 58), thus providing a mechanism by which abundant, high-affinity binding proteins such as ABF1 and RAP1 can outcompete histones for their binding sites without assistance by accessory proteins and therefore, by implication, without an activation domain (27).

Although both ABF1 and RAP1 could perturb chromatin structure via nucleosomal binding sites, there were some subtle differences in the structural changes elicited by the two proteins, as seen in the slightly different MNase cleavage patterns (Fig. 1). We do not at present understand the basis for this difference, although it does not appear to involve the C-terminal regions nor proteins interacting with these regions, since the MNase cleavage patterns induced by ABF1 and RAP1 do not depend on these regions (Fig. 3) (59). It also seems likely that these subtle differences will not, at least in most cases, lead to substantial differences between ABF1 and RAP1 in facilitating transcriptional activation, as the two function essentially interchangeably at the *HIS4*, *HIS7*, and *ADE5,7* promoters. At these promoters, both ABF1 and RAP1 were able to synergize with either GCN4 (*HIS4*, *HIS7*) or BAS1/BAS2 (*ADE5,7*), consistent with a general function of opening chromatin to facilitate activator access. This notion is also supported by recent work showing that the region of the *HIS7* promoter surrounding the ABF1 binding site is free of nucleosomes (53) and with the finding that even heterologous DNA-binding proteins expressed ectopically in yeast can facilitate GCN4 binding to nearby sites (25). Furthermore, ABF1 was able to facilitate GCN4-mediated activation at *HIS4* even when lacking its transactivation domain, consistent with previous observations for RAP1 (59) (Fig. 4B). Taken together, these experiments and previous work point to ABF1 and RAP1 being prototypes of a category of DNA-binding proteins that mark chromatin by outcompeting histones for their binding sites and creating an open region of chromatin which other either less-abundant or lower-affinity DNA-binding proteins may then access. It seems likely that other proteins play this same role both in yeast (e.g., REB1, another GRF) and in higher eukaryotes (31). Such proteins, if identified in higher eukaryotes, could potentially be useful in maintaining open chromatin in transgenic applications.

We observed a notable and surprising difference between ABF1 and RAP1 when we tested whether continuous occupancy by ABF1 at the modified *HIS4* promoter was required for ongoing transcription. As mentioned above, transcription of several ABF1-driven promoters (i.e., promoters containing ABF1 binding sites that had been shown to contribute to activation) had been reported to be unchanged upon loss of ABF1 binding in an *abf1-1 ts* mutant (44). In contrast, we had

found that GCN4-mediated *HIS4* activation greatly decreased upon loss of RAP1 binding (59). This contrasting behavior could have been due to differences in promoter structure rather than differences in ABF1 and RAP1 function. However, we found in a direct comparison at the *HIS4* promoter that ABF1 could contribute to activation even without continuous binding, whereas continuous binding by RAP1 was required for it to facilitate activation (Fig. 4A and 6). ChIP demonstrated that ABF1 was lost to a quantitatively similar degree at *HIS4* and *SPT15* in the *abf1-1 ts* mutant at 37°C (Fig. 7). Since previous work had shown quantitative loss of ABF1 from the *SPT15* promoter under these conditions, this suggests that ABF1 similarly is mostly or completely lost from the modified *HIS4* promoter. We suggest that, in contrast to RAP1, ABF1 changes the promoter in a way that is stable to loss of ABF1 and that facilitates transcriptional activation. The most obvious possibility is that ABF1 modifies chromatin, either directly or via recruitment of chromatin-modifying enzyme(s), in a way different from that of RAP1. Future work will be directed at testing this possibility.

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